Distribution and larval habitats of *Anopheles* species in northern Gyeonggi Province, Republic of Korea

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ABSTRACT: A total of 180 larval collection sites (e.g., rice paddies, marshes, ground pools, ponds, stream margins, and irrigation and drainage ditches) was surveyed within a 2 km radius from Warrior Base training area, 5 km south of Panmunjeom (Joint Security Area, demilitarized zone), Gyeonggi Province, Republic of Korea (ROK), from May through October, 2007 to characterize larval habitat distributions of members of the *Anopheles* Hyrcanus Group (*An. sinensis, An. lesteri, An. pullus, An. belenrae*, *An. kleini,* and *An. sineroides*). A total of 5,859 anopheline larvae was collected from 84.4% of the sites surveyed, of which 4,071 were identified to species by polymerase chain reaction (PCR) using the ribosomal DNA internal transcribed spacer 2 (rDNA ITS2). *Anopheles sinensis* (52.6%) was the most frequently collected, followed by *An. kleini* (29.4%), *An. sineroides* (9.8%), *An. pullus* (6.7%), *An. belenrae* (1.1%), and *An. lesteri* (0.5%). *Anopheles pullus* and *An. kleini* were collected in greater proportions in May and from May – July, respectively. Few *An. sinensis* were collected from May – June, but it was the predominant species collected by August, and accounted for >80% of all larvae from September – October. *Anopheles kleini* was found in all habitats sampled; however, it was collected most frequently in young growth rice paddies, while *An. sinensis* was collected more frequently in mature and post-harvest paddies. *Anopheles pullus* was associated with pre-cultivated rice paddies, including water-filled tire ruts left from the previous fall's harvest. ***Journal of Vector Ecology*** 36 (1): 124-134. 2011.

Keyword Index: Distribution, habitats, *Anopheles,* Munsan, Gyeonggi Province, Republic of Korea (ROK).

INTRODUCTION

During the Korean War, the lack of both vector-borne disease detection capability and integration of appropriate interventions greatly affected casualty figures, which resulted in more than 6,000 cases of vivax malaria diagnosed in Korea and an estimated 12,000 cases among soldiers that returned to the U.S. after exposure in Korea (Fritz and Andrews 1953, Hankey et al. 1953, Pruitt 1954). Following the Korean War, malaria continued to pose a serious health threat and impacted military readiness. As a result of malaria control efforts, in 1979 the World Health Organization declared the Republic of Korea (ROK) malaria free (WHO 1981). In 1993, *vivax* malaria reemerged along the demilitarized zone (DMZ), rapidly spread throughout northern Gyeonggi and Gangwon Provinces where U.S. Forces Korea (USFK) conducts military training, and again posed a serious health threat that impacted military readiness (Chai et al. 1994, Coleman et al. 2002, Park et al. 2003, Kim et al. 2007, 2009b, Klein et al. 2009). U.S. military training sites and installations near the DMZ, location of almost all reported U.S. military malaria cases, are often co-located with wetland rice farming (the primary agriculture in the ROK) and associated ditches, ponds, and irrigation canals (Kim et al. 2009a, Klein et al. 2009). Larval mosquito control is not practiced and alternate flooding and drying of rice paddies as a control measure is made difficult due to frequent heavy rains during the wet monsoon season. Furthermore, seasonal and annual environmental factors impact larval and adult mosquito populations and malaria transmission. For example, brief periods of low rainfall, which result in drying of rice paddies where irrigation is lacking, adversely impact larval and subsequently adult populations through loss of larval habitat. In addition, the early onset of spring and/or late onset of fall temperatures increase the duration of the mosquito breeding season and malaria transmission. The late onset of spring delays the beginning of the malaria season, while the early onset of cold temperatures curtails the malaria season.

Due to the potential medical impact of malaria on U.S. Forces Korea (USFK), the Eighth U.S. Army and the U.S. Air Force conduct mosquito-borne disease control programs, which include mosquito surveillance that identifies the geographical distribution and relative density of potential vector populations (Kim et al. 2007, Kim et al. 2010). The recent clarification of the *An.* Hyrcanus Group (Wilkerson

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14. ABSTRACT
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et al. 2003, Li et al. 2005) and subsequent naming of two species, namely An. kleini and An. belenrae (Rueda 2005), allow for more accurate data collection, including the geographical distribution and population densities of specific vectors (Rueda et al. 2006).

An effective and efficient surveillance system must include all three components, the human, parasite, and entomological aspects, to have the greatest predictive value for reducing the disease. Often, the primary focus is on human populations and malaria infection rates, while the entomological component, which is designed to better understand the dynamics of larval mosquito habitat associations, relative adult populations, and transmission of mosquito-borne diseases, is neglected (Russell 1963). The objectives of this study were to determine the relative larval species composition and population densities, habitat preferences based on species composition and relative population densities, and seasonal changes in relative population densities. Data included herein provide preliminary information on the relative seasonal and habitat distribution of larval *Anopheles* spp. populations near the DMZ.

MATERIALS AND METHODS

Study site

Warrior Base is a primary U.S. training hub where military personnel are currently housed in air-conditioned barracks (as compared to ill-kept large tents where they were housed prior to 2007) while conducting field training exercises at nearby firing ranges, maneuver sites, and small to moderately sized tactical cantonment sites and a large multipurpose range (Story Range) (Figure 1). It is adjacent to the ROK military base and located approximately 3 km south of Panmunjeom (Joint Security Area), Gyeonggi Province, which borders a 4 km-wide DMZ that separates the ROK from the Democratic People's Republic of Korea (DPRK, North Korea), and is 1.5 km east of Tongilchon, 1 km north of the Imjin River, and 10 km north of Munsan. The area surrounding Warrior Base is characterized by relatively narrow stream valleys with associated wetland rice agriculture, drainage and irrigation ditches, ponds, and marshes (low-lying areas and fallow rice paddies) bounded by forested hills (Rueda et al. 2010). Several moderate to fast flowing streams, which fill after heavy rains, intersect the area and drain into the Imjin River. Numerous earthen and concrete irrigation and drainage ditches with slow to fast moving water, depending on precipitation and release of water for irrigation, are distributed throughout the rice growing areas. Ponds (5-15 m in diameter), earthen and concrete ditches, rice paddies, ground pools/grassy depressions, stream margins, and marshes within a 2-km radius of Warrior Base cantonment area were surveyed. Pond water levels were variable as a result of irrigation of rice paddies during periods when rainfall was insufficient. During May, rice paddies were in various stages of preparation for planting (untilled to partially or completely tilled) and flooding. By June, all rice paddies were tilled in preparation for planting or were planted in rice. Several low-lying grassy marshes and grassy depressions were surveyed adjacent to rice paddies and were dependent upon rainfall for flooding. Two streams were surveyed, one being very limited in vegetation along the margin and the other with tall grasses that frequently flooded as a result of release of water for irrigation and periodic heavy rains.

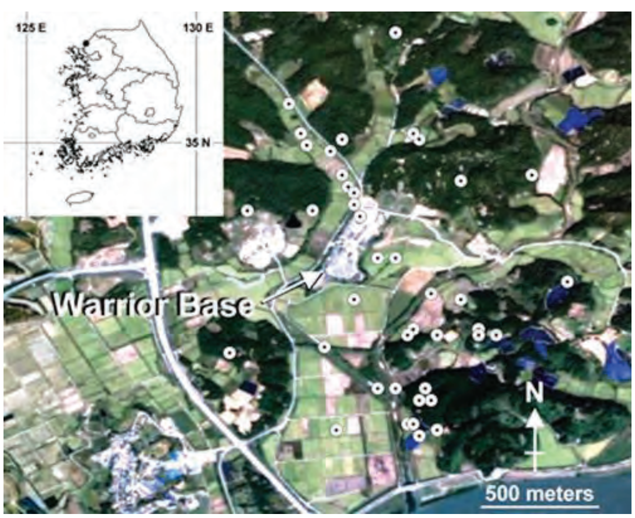

Figure 1. Warrior Base and surrounding area where monthly larval mosquito surveys were conducted (circle) from May – October, 2007.

Larval collection and identification by polymerase chain reaction

Surveyed habitats were classified as (1) rice paddies (including untilled, tilled, planted, and harvested rice paddies), (2) irrigation and drainage ditches, (3) ponds of various sizes, (4) marshes (fallow rice paddies), (5) grassy ground pools and man-made depressions, and (6) stream pool and stream margins.

Monthly larval surveillance was conducted near Warrior Base from May through October, 2007 as described by Rueda et al. (2010). Briefly, each site was sampled using a standard dipper (350 ml, 13 cm diameter) or a white plastic tray (25 x 20 x 4 cm) (BioQuip, Rancho Dominquez, CA) up to one h or until 100 *Anopheles* larvae were collected. Larvae were placed in plastic Whirl-Pak® bags (118 ml, 8 x 18 cm; BioQuip, Rancho Dominquez, CA) and transported to the 5^{th} Medical Detachment where they were transferred to 2 ml cryovials containing 100% ethyl alcohol or reared to the adult stage and mounted on paper points in accordance with standard practices (Tanaka et al. 1979, Lee 1998, Rueda et al. 2010). Data from each collection were recorded on a modified standard form (WRBU 2010) that included a detailed habitat description, photographs of each collection site, unique collection site number, date of collection, and latitude and longitude, and numbers, genus, and species of mosquitoes (particularly culicines) for each collection site. Curated adults and larvae, with electronic collection records, were shipped to WRBU where adult specimens, mounted exuviae, and collection records were deposited in the U.S. National Museum of Natural History, Smithsonian

Table 1. Monthly number (%) of larval sites containing water, number (mean) of larvae collected, number (%) assayed and identified to species, and number (%) of *Anopheles* larvae collected, by species, for each survey period at Warrior Base, Republic of Korea, from May – October, 2007.

Month	No. sites surveyed	No. (%) sites w/ water	No. (%) sites w/ larvae	No. (Mean) larvae[1]	No. (%) *Anopheles* tested	*An. sinensis*[2]	*An. kleini*	*An. pullus*	*An. sineroides*	*An. belenrae*	*An. lesteri*
May	36	36 (100)	23 (63.9)	271 (7.5)	206 (76.0)	12 (5.8)	76 (36.9)	66 (32.0)	32 (15.5)	20 (9.7)	0
June	26	26 (100)	22 (84.6)	670 (25.8)	473 (70.6)	65 (13.7)	197 (41.6)	80 (16.9)	128 (27.1)	1 (0.2)	2 (0.4)
July	29	29 (100)	27 (93.1)	1,162 (40.1)	754 (64.9)	292 (38.7)	418 (55.4)	35 (4.6)	5 (0.7)	2 (0.3)	2 (0.3)
August	33	32 (97.0)	30 (93.8)	1,380 (43.1)	1,123 (81.4)	644 (57.3)	345 (30.7)	47 (4.2)	69 (6.1)	8 (0.7)	10 (0.9)
September	35	29 (82.9)	26 (89.7)	1,308 (45.1)	767 (58.6)	486 (63.4)	128 (16.7)	30 (3.9)	110 (14.3)	9 (1.2)	4 (0.5)
October	37	28 (75.7)	24 (85.7)	1,068 (38.1)	748 (70.0)	644 (86.1)	32 (4.3)	14 (1.9)	54 (7.2)	3 (0.4)	1 (0.1)
Total	196	180 (91.8)	152 (84.4)	5,859 (32.6)	4,071 (69.5)	2,143 (52.6)	1,196 (29.4)	272 (6.7)	398 (9.8)	43 (1.1)	19 (0.5)

Institution, Suitland, MD. DNA was isolated from individual *Anopheles* larvae and adults (one or two legs/adult) for PCR identification (Wilkerson et al. 2003, Li et al. 2005).

RESULTS

Percent of *Anopheles* collected

A total of 196 sites classified into six primary habitats was surveyed, of which 91.8% contained water at the time of the survey (Table 1). Of the 180 sites with water, 84.4% were positive for *Anopheles* larvae. From a total of 5,859 larvae collected, 69.5% were identified by PCR to species (Wilkerson et al. 2003, Li et al. 2005). Overall, *An. sinensis* Wiedemann accounted for 52.6% of all larvae identified, followed by *An. kleini* Rueda (29.4%), *An. sineroides* Yamada (9.8%), *An. pullus* Yamada (6.7%), *An. belenrae* Rueda (1.1%), and *An. lesteri* Baisas and Hu (0.5%). Two species of *Anopheles* (*An. koreicus* Yamada and Watanabe and *An. lindesayi japonicus* Giles) present in Korea (Sames et al. 2008) were not collected. *Anopheles kleini* accounted for the highest proportion of mosquitoes collected from May through July (range 36.9%-55.4%), while decreasing to a low of 4.3% by October (Figure 2A). The highest proportions of *An. pullus* (32.0%) and *An. belenrae* (9.7%) were observed in May and decreased thereafter to a low of 1.9% and 1.1%, respectively, by October. In contrast, the proportion of *An.* accounted for 5.8% of all larvae identified in May, but increased throughout the season to a high of 86.1% by October. The proportion of *An. sineroides* collected varied throughout all collection periods from a high 27.1% in June to a low of 0.7% in July. Low numbers of *An. lesteri* larvae were collected throughout the survey period, with the highest proportion collected during August (0.9%).

Rice paddies (1,335 larvae), ponds (1,134 larvae), and drainage/irrigation ditches (1,090 larvae) accounted for the majority of anopheline larvae collected and identified (3,559/4071; 87.4%) (Table 2). *Anopheles sinensis* accounted for the highest proportion of all larvae (36.4% – 58.6%) for all habitats, with the exception of stream margins where *An. kleini* accounted for the highest proportion (Figure 2B). *Anopheles kleini* accounted for the second highest proportion of mosquitoes collected from all habitats, except for depressions, where *An. sineroides* comprised the highest proportion. The highest proportion of *An. pullus* was observed for marsh habitats (14.6%) and was uniformly present in other habitats (3.9% to 7.7%).

Mean number of *Anopheles* collected

Overall, larval mean numbers rapidly increased for all collection sites from a low of 5.7 in May to a high of 35.1 by August, shortly after the end of the rainy monsoon season, and remained relatively stable from September – October (range, 26.4-27.7) (Table 2, Figure 3A). In general, numbers of larvae for all habitats, except stream margins, followed that of the overall mean. The mean number of larvae collected from rice paddies (27.8), marshes (28.0), and ponds (24.1) were similar (Table 2). Ground pools/depressions were infrequently observed, but following the monsoon rains in August, high mean numbers (34.0) of larvae were collected in these habitats. Few larvae were collected along the slow to fast flowing grassy stream margins and pools that flooded frequently.

Overall, the mean numbers of *An. sinensis* larvae for all habitats, except streams, were higher than the other species (Table 3). The mean numbers of *An. kleini* larvae were highest for stream margins and it was the second most commonly collected for all other habitats, except ground pools/depressions where *An. sineroides* was more frequently collected. For all habitats, *An. sinensis* mean larval populations were very low in May (0.5/site) (Figure 3B). Mean numbers increased through August (21.5/site) at the end of the wet monsoons and declined in September (18.7/site) as habitats dried up due to rice harvesting. While habitats were limited after harvesting in October, mean numbers increased to a high of 26.8/site. Mean numbers of *An. kleini* larvae per site increased from 3.3 to 16.1 in July and decreased thereafter to a low of 1.3 in October. Mean numbers of *An. pullus* per site increased from 2.9 to 3.3 in June and remained low throughout the remainder of the season (range 0.6 – 1.6) (Figure 3B).

Rice paddies provided the largest area for mosquito breeding near Warrior Base (Figure 1) and accounted for 32.8% (mean 27.8 larvae/site) of all *Anopheles* spp. identified by PCR (Table 2). Overall, *An. sinensis* was the

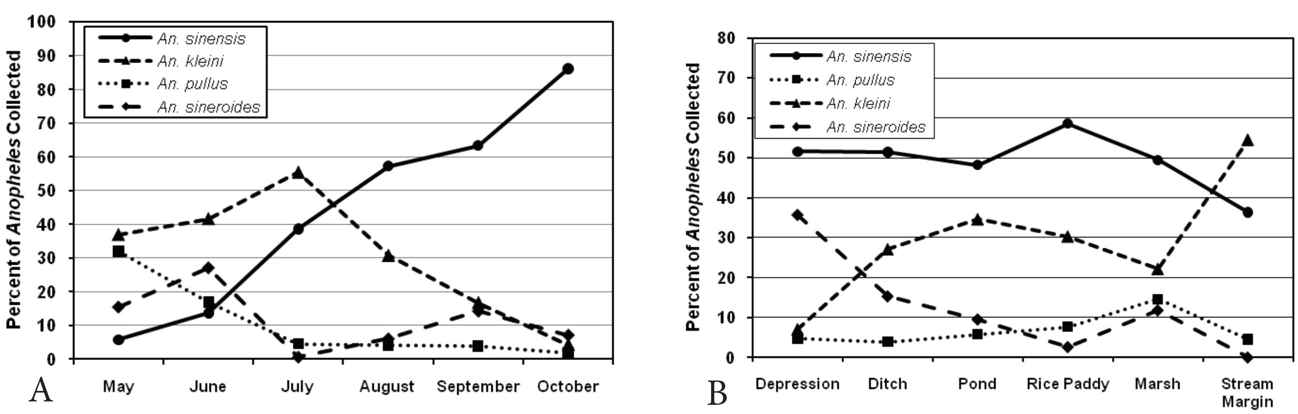

Figure 2. Percent of *Anopheles* spp. collected monthly (A) and selected habitats (B) near Warrior Base from May – October, 2007.

Table 2. Monthly number (mean) of anopheline larvae collected and identified from selected habitats near Warrior Base, Republic of Korea, from May – October, 2007.

Habitat	Sites w/ Water	Number (%) w/ larvae	Number larvae collected	Number (%) larvae identified	May	Jun	Jul	Aug	Sep	Oct	Total (Mean)
Rice Paddy	48	41 (85.4)	1,922	1,335 (69.5)	81 (6.8)	227 (32.4)	309 (30.9)	223 (27.8)	235 (39.1)	260 (52.0)	1,335 (27.8)
Ditch	56	46 (82.1)	1,773	1,090 (61.5)	43 (4.3)	47 (7.8)	179 (25.6)	401 (36.5)	311 (25.9)	109 (10.9)	1,090 (19.5)
Pond	47	42 (89.4)	1,530	1,134 (74.1)	37 (5.3)	127 (15.9)	202 (25.3)	336 (42.0)	169 (21.1)	263 (32.9)	1,134 (24.1)
Marsh	14	12 (85.7)	390	364 (93.3)	33 (8.3)	43 (21.5)	40 (20.0)	95 (47.5)	41 (41.0)	112 (56.0)	364 (28.0)
Ground Pool	9	8 (88.9)	211	126 (59.7)	10 (5.0)	29 (14.5)	15 (15.0)	68 (34.0)	0[1]	4 (4.0)	126 (14.0)
Stream Margin	6	3 (50.0)	33	22 (66.7)	2 2.0)	0[1]	9 (9.0)	0[1]	11 (11.0)	0[1]	22 (3.7)
Total (Mean)	180	152 (84.4)	5,859	4,071 (69.5)	206 (5.7)	473 (18.2)	754 (26.0)	1,123 (35.1)	767 (26.4)	748 (27.7)	4,071 (22.7)

Table 3. Number (mean) of *Anopheles* spp. collected and identified from different habitats near Warrior Base, Republic of Korea, May – October, 2007.

Habitat	Total sites surveyed	Sites (%) w/water	No. (%) sites w/ larvae	No. larvae[1]	No. (%) *Anopheles* Tested[2]	*An. sinensis*	*An. kleini*	*An. pullus*	*An. sineroides*	*An. belenrae*	*An. lesteri*
Rice Paddy	59	48 (81.4)	41 (85.4)	1,922	1,335 (69.5)	782 (16.3)	405 (8.4)	103 (2.2)	35 (0.7)	7 (0.2)	3 (0.1)
Ditch	58	56 (96.6)	46 (82.1)	1,773	1,090 (61.5)	561 (10.0)	296 (5.3)	43 (0.8)	167 (3.0)	17 (0.3)	6 (0.1)
Pond	47	47 (100.0)	42 (89.4)	1,530	1,134 (74.1)	547 (11.6)	393 (8.4)	66 (1.4)	108 (2.3)	17 (0.4)	3 (0.1)
Ground Pool	11	9 (81.8)	8 (88.9)	211	126 (59.7)	65 (7.2)	9 (1.0)	6 (0.7)	45 (5.0)	0	1 (0.1)
Marsh	15	14 (93.3)	12 (85.7)	390	364 (93.3)	180 (12.9)	81 (5.8)	53 (3.8)	43 (3.1)	1 (0.1)	6 (0.4)
Stream Margin	6	6 (100.0)	3 (50.0)	33	22 (66.7)	8 (1.3)	12 (2.0)	1 (0.2)	0	1 (0.2)	0
Total (Mean)	196	180 (91.8)	152 (84.4)	5,859	4,071 (69.5)	2,143 (11.9)	1,196 (6.6)	272 (1.5)	398 (2.2)	43 (0.2)	19 (0.1)

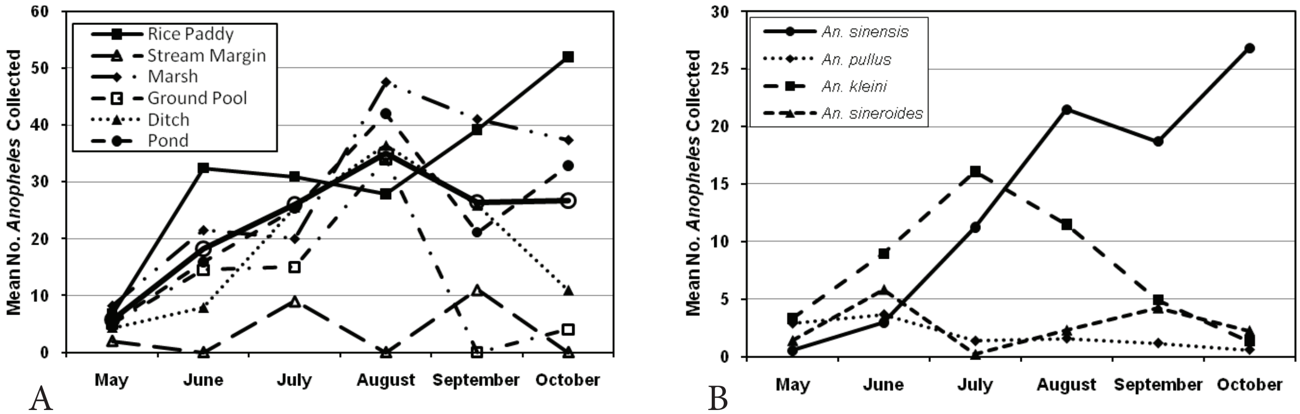

Figure 3. Mean number of *Anopheles* spp. collected from selected larval habitats (A) and all habitats (B) near Warrior Base from May – October, 2007.

most frequently collected in rice paddies throughout the collection period (58.6% of all larvae), increasing from a low of 0.8 in May (untilled, partially tilled, and tilled-not planted) to a high of 47.4 mean number of larvae by October (young-mature and harvested rice crops) (Table 4, Figure 4A). This was followed by *An. kleini* (16.3), *An. pullus* (2.2), *An. sineroides* (0.7), *An. belenrae* (0.2), and *An. lesteri* (0.1) (Table 3). In contrast, *An. kleini* populations increased from a low of 1.3 to a high of 21.7 in June and declined thereafter to a low of 1.0 by October (Figure 4A). *Anopheles pullus* were most frequently collected from tilled rice paddies during May, with low numbers collected during June through October.

Overall, *An. sinensis* accounted for 51.5% of all *Anopheles* spp. collected from drainage/irrigation ditches, with mean numbers increasing from a low in May (0.2) to a high in August (23.1) and with similar numbers recorded for July, September, and October (12.1-13.1) (Figure 4B). *Anopheles kleini* accounted for 27.2% of all anopheline larvae collected with the highest mean number observed in July (15.5) and August (11.4). *Anopheles sineroides* accounted for 15.3% of all anophelines identified but was irregularly collected with the highest mean numbers in June (5.5) and September (7.6). Relatively low numbers of *An. pullus* (0.3 – 1.4), *An. belenrae* (0.0 – 1.6), and *An. lesteri* (0.0 – 0.5) were collected for all periods.

Small (3 m in diameter) to relatively large (15 m in diameter) ponds were broadly scattered throughout the area and accounted for 27.9% (mean 24.1/pond) of all larvae identified. Overall, *An. sinensis* (11.6) accounted for the highest mean number of larvae collected through the collection period from the ponds, followed by *An. kleini* (8.4), *An. sineroides* (2.3), *An. pullus* (1.4), *An. belenrae* (0.4), and *An. lesteri* (<0.1) (Table 3). Overall, *An. sinensis* larval populations were very low for ponds from May-June (0.7-1.0), but rapidly increased by August (21.4) and peaked again after harvesting (30.0) (Figure 4C).

Marsh-like (fallow rice paddies) areas (8.9%), grassy depressions/excavated ground pools (3.1%), and stream margins (0.5%) accounted for only12.6% of all *Anopheles* spp. identified by PCR (Table 3). Mean numbers of each species collected from marshes were variable as a result of periodic rains and relative seasonal abundance of each species (Figure 4D). While grassy depressions followed a similar pattern, they were dependent upon rainfall and were dry during some surveys (e.g., September) (Table 2). Streams/large irrigation canals were influenced by rains and release of water for irrigation, alternating with moderate to fast flowing water which likely washed many of the larvae downstream. Larvae were not collected during three of the six surveys (Table 2).

Rice paddies were surveyed in various stages of preparation for planting through harvesting (Table 4). *Anopheles pullus* (mean 4.1) was the most frequently collected from untilled, partially tilled, and tilled in preparation for planting, while *An. sinensis* (mean 18.6) and *An. kleini* (mean 11.9) were more frequently collected from young to mature rice paddies. *Anopheles sinensis* (93.8%, mean 55.0) was nearly exclusively collected from harvested rice paddies during October.

DISCUSSION

Vivax malaria is endemic in the ROK with 405 malaria cases reported from 1993-2007 in U.S. (n = 361) and Korean Augmentation Troops to the United States Army (KATUSA) soldiers (n = 43) and civilians (n = 1), with the majority attributed to exposure at Warrior Base and associated training sites within 5-km south of Warrior Base (Kim et al. 2009a, Klein et al. 2009). Past reports of relative abundance, distributions, and malaria vector status have raised many questions as *Anopheles* spp. were recently synonymized and new species identified in the ROK (Wilkerson et al. 2003, Li et al. 2005, Rueda 2005). These molecular studies determined that some members of the *Anopheles* Hyrcanus Group in Korea cannot be identified using known morphological characteristics. Therefore, previous and continued current studies based solely on morphological characters need to be reevaluated to clarify the bionomics and vector status of *An. sinensis* s.s., previously thought to be the primary vector of malaria in the ROK, and the other four members of the *Anopheles* Hyrcanus Group in Korea. The mosquito surveillance program was expanded to include larval surveillance in 2007, as reported here, near Warrior Base,

Table 4. Total number (mean number) of *Anopheles* spp. collected from rice paddies in various stages of development at Warrior Base, Republic of Korea, from May – October, 2007.

Rice Paddy Characteristics	Number Sites	*An. sinensis*	*An. kleini*	*An. pullus*	*An. sineroides*	*An. belenrae*	*An. lesteri*
Untilled	1	2 (2.0)	2 (2.0)	2 (2.0)	0	0	0
Partially Tilled	3	3 (1.0)	5 (1.7)	26 (8.7)	3 (1.0)	1 (0.3)	0
Tilled-Not Planted	8	0	1 (0.1)	21 (2.6)	14 (1.8)	1 (0.1)	0
Young-Mature Rice	33	612 (18.6)	392 (11.9)	52 (1.6)	16 (0.5)	3 (0.1)	3 (0.1)
Harvested Rice	3	165 (55.0)	5 (1.7)	2 (0.7)	2 (0.7)	2 (0.7)	0
Total (Mean)	48	782 (16.3)	405 (8.4)	103 (2.2)	35 (0.7)	7 (0.2)	3 (<0.1)

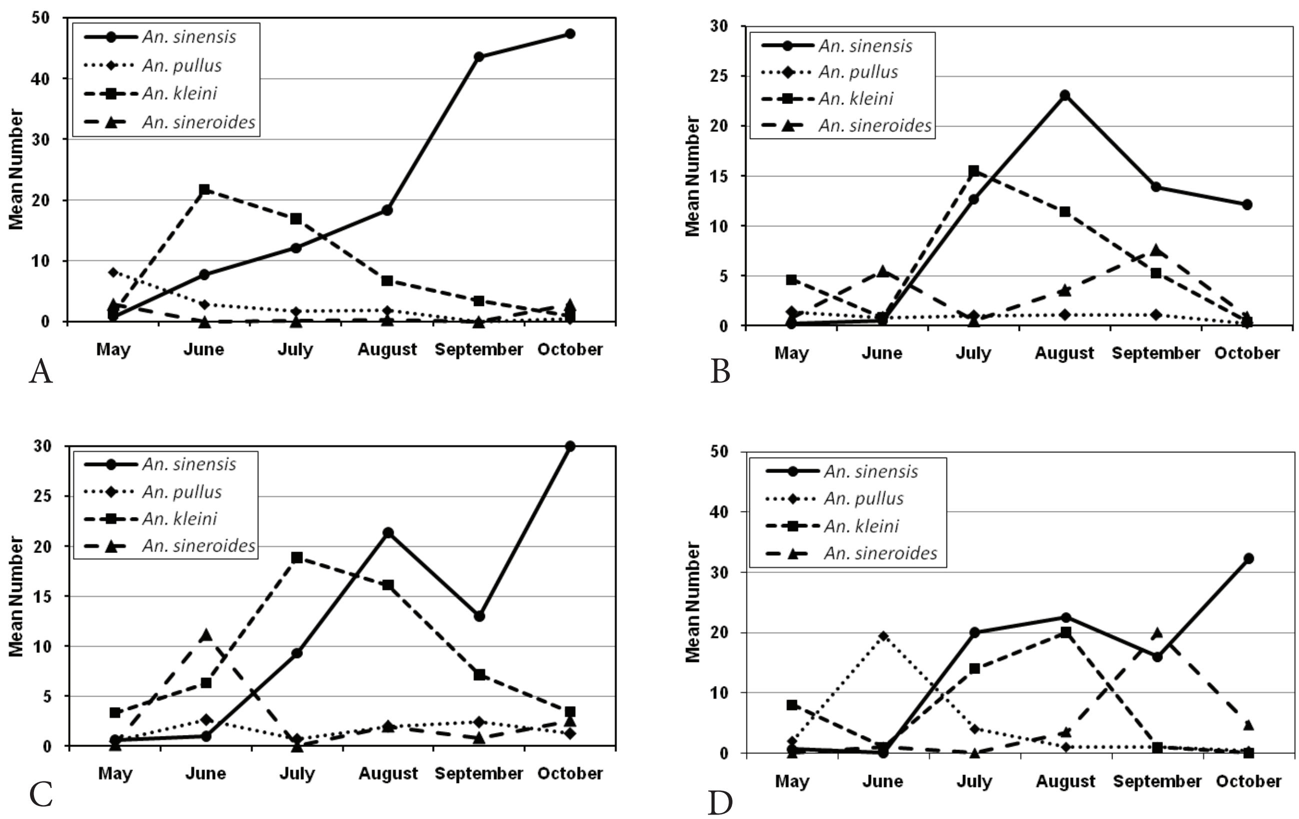

Figure 4. Mean number of *Anopheles* spp. collected from rice paddies (A), irrigation ditches with grassy margins (B), ponds (10-15 m in diameter) (C), and marshes (D) near Warrior Base from May – October, 2007.

a malaria high-risk site where U.S. and ROK soldiers and associated civilians train or reside, to identify ecological parameters that impact on malaria vector populations, including habitat preferences.

Anopheles sinensis, considered a secondary malaria vector by some (Lee et al. 2007, Joshi et al. 2009), is the most commonly collected mosquito throughout Korea (Kim et al. 2007, Rueda et al. 2010). In northern Gyeonggi and northwestern Gangwon Provinces near the DMZ, where it is estimated that >80% of transmission of *vivax* malaria cases are reported, *An. sinensis* accounted for 56.2% of adult *Anopheles* Hyrcanus Group adults captured in Paju (near the DMZ), while *An. kleini* and An. *pullus*, the primary vectors of *vivax* malaria in Korea, accounted for 40.0% and 3.0%, respectively (Rueda et al. 2006, Kim et al. 2007, Lee et al. 2007, Rueda et al. 2010). However, in malaria low-risk areas south of Seoul, *An. sinensis* accounted for 94.7% – 97.2% of all adult *Anopheles* spp. collected (Kim et al. 2007, Lee et al. 2007). Similarly, larval collections that were conducted concurrently at Jimbo and Hayang, Gyeongsangbuk Province (southeastern Korea) showed that *An. sinensis* larvae accounted for >90% of all *Anopheles* larvae collected and that changes in their population densities followed that observed at Warrior Base (Rueda et al. 2010).

The percentage of adult *An. kleini* was highest during the late spring and early summer, while the proportion of *An. sinensis* adults collected was highest during the late summer and fall at both Tongilchon and Majeong-ri in Paju (Kim et al. 2007). These results are similar to changes observed over time in the proportion of larvae collected near Warrior Base in 2007. While peak larval populations of *An. kleini* were observed during July at Warrior Base, peak larval populations (although low) were reported during August at Hayang, in the south (Rueda et al. 2010). At Warrior Base, adult *An. pullus* populations were highest in May and June but rapidly decreased from July through October, while the highest proportion of larvae collected were observed during May (32.0%) followed by a rapid decline in June that continued throughout the mosquito season. These findings were similar to those reported by Rueda et al. (2010) that showed that *An. pullus* populations were highest during May at both Jinbo and Hayang.

Both adult *An. belenrae* and *An. lesteri* made up a small proportion of adults collected in New Jersey light traps and Mosquito Magnets® and similarly, few larvae were collected throughout this study and surveillance at Jinbo and Hayang (Lee et al. 2007, Kim et al. 2007, Rueda et al. 2010).

Population shifts in the proportion and abundance of *Anopheles* spp. is likely influenced by environmental factors such as, (1) the onset of monsoon rains that are highly variable annually, (2) occasional typhoons, (3) the maturation of vegetation bordering streams, ditches, and rice paddies, (4) tilling, planting, maturation of rice plants, and harvesting, (5) intermittent flooding and drying of rice crops, especially those areas dependent upon rain, (6) farming practices, such as cutting of vegetation along ditches and earthen banks separating rice paddies and use of fertilizers and insecticides, and (7) interspecific competition. Rice paddies account for the largest area of agricultural lands and changes to the rice paddy over the mosquito season appear to heavily impact the distributions and densities of larval and adult populations. For example, *An. pullus* was collected in higher proportions from freshly tilled rice paddies than other species, while *An. sinensis* populations exceeded 90% of all *Anopheles* spp. collected from harvested rice paddies. Although *An. kleini* and *An. pullus*, considered to be the primary malaria vectors near the DMZ, were collected over a wide range of larval habitats, including rice paddies, ditches, small ponds, and marshes, their relative high abundance near the DMZ and relative low abundance south of Seoul is difficult to explain as habitats and farming practices appear to be similar throughout the ROK.

Mosquito populations are also impacted by winter survival, as overwintering adults and/or eggs impact on initial populations resulting from extreme periods of cold and snow cover. Early or late onset of spring and fall temperatures impact larval and adult populations, especially during the course of the mosquito season. For example, during one year, the late onset of fall temperatures resulted in high larval populations during the first week of November, while during another year, the early onset of fall temperatures resulted in few larvae collected near the end of October (T.A. Klein, unpublished data).

To develop and implement comprehensive malaria control programs, knowledge of vector and non-vector identification and bionomics is essential, including their relative geographical distributions, population densities, and larval habitats (Russell 1963). In the ROK, habitat and species association data are complicated, as water from streams is channeled into irrigation ditches and canals, while overflow water flows back into irrigation and drainage ditches into streams, and finally into major river systems. For example, *An. lindesayi japonicus* larvae are occasionally collected in rice paddies, but 1st and 2nd instars are most frequently collected in shaded mountain stream pools and eddies, and during heavy rains some get caught in the currents and are flushed into valley streams, irrigation canals, and rice paddies (Sames et al. 2008, Kim et al. 2009b, Rueda et al. 2010). Therefore, to better define habitat preferences for oviposition, extensive larval collections that identify breeding sites based on stages of larval development are planned.

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